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## Mutation analysis in Bardet–Biedl syndrome by DNA pooling and massively parallel resequencing in 105 individuals

**Sabine Janssen,**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Gokul Ramaswami,**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Erica E. Davis,**

Center for Human Disease Modeling, Duke University Medical Center, Durham, NC, USA.

Department of Pediatrics, Duke University Medical Center, Durham, NC, USA

**Toby Hurd,**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Rannar Airik,**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Jennifer M. Kasanuki,**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Lauren Van Der Kraak,**

Department of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada

**Susan J. Allen,**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Philip L. Beales,**

Molecular Medicine Unit, UCL Institute of Child Health, London, UK

**Nicholas Katsanis,**

Center for Human Disease Modeling, Duke University Medical Center, Durham, NC, USA.

Department of Pediatrics, Duke University Medical Center, Durham, NC, USA. Cell biology, Duke University Medical Center, Durham, NC, USA

**Edgar A. Otto, and**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Friedhelm Hildebrandt**

Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA.

Howard Hughes Medical Institute, Chevy Chase, MD, USA. University of Michigan Health System, 8220C MSRB III, 1150 West Medical Center Drive, Ann Arbor, MI 48109-5646, USA

Friedhelm Hildebrandt: fhilde@umich.edu

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Correspondence to: Friedhelm Hildebrandt, fhilde@umich.edu.

S. Janssen and G. Ramaswami contributed equally to this work.

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## Abstract

Bardet–Biedl syndrome (BBS) is a rare, primarily autosomal-recessive ciliopathy. The phenotype of this pleiotropic disease includes retinitis pigmentosa, postaxial polydactyly, truncal obesity, learning disabilities, hypogonadism and renal anomalies, among others. To date, mutations in 15 genes (*BBS1–BBS14*, *SDCCAG8*) have been described to cause BBS. The broad genetic locus heterogeneity renders mutation screening time-consuming and expensive. We applied a strategy of DNA pooling and subsequent massively parallel resequencing (MPR) to screen individuals affected with BBS from 105 families for mutations in 12 known *BBS* genes. DNA was pooled in 5 pools of 21 individuals each. All 132 coding exons of *BBS1–BBS12* were amplified by conventional PCR. Subsequent MPR was performed on an Illumina Genome Analyzer II™ platform. Following mutation identification, the mutation carrier was assigned by CEL I endonuclease heteroduplex screening and confirmed by Sanger sequencing. In 29 out of 105 individuals (28%), both mutated alleles were identified in 10 different *BBS* genes. A total of 35 different disease-causing mutations were confirmed, of which 18 mutations were novel. In 12 additional families, a total of 12 different single heterozygous changes of uncertain pathogenicity were found. Thus, DNA pooling combined with MPR offers a valuable strategy for mutation analysis of large patient cohorts, especially in genetically heterogeneous diseases such as BBS.

## Introduction

Bardet–Biedl syndrome (BBS; OMIM# 209900) is a clinically pleiotropic disorder caused by defects of primary cilia (Zaghloul and Katsanis 2009). The cardinal diagnostic criteria are retinitis pigmentosa, postaxial polydactyly, truncal obesity, learning disabilities, abnormalities of the urogenital tract and renal anomalies (Baker and Beales 2009; Bardet 1920; Biedl 1922). Moreover, a large spectrum of secondary features can occur, which have been updated in a recent review by Baker and Beales (2009).

The prevalence of BBS is low in the general population, ranging from 1:125,000 to 1:160,000 in Europe (Beales et al. 1997; Haim 1992; Klein and Ammann 1969), and 1:65,000 in Arab populations (Farag and Teebi 1988). A higher incidence is found in certain isolated populations, such as those in Newfoundland, Kuwait and the Faroe islands (Hjortshøj et al. 2010; Moore et al. 2005; Teebi 1994).

To date, mutations in 15 genes (*BBS1–BBS12*, *MKSI*, *CEP290* and *SDCCAG8*) have been shown to cause BBS under an autosomal recessive mode of inheritance (Ansley et al. 2003; Badano et al. 2003a; Chiang et al. 2004, 2006; Katsanis et al. 2000; Leitch et al. 2008; Li et al. 2004; Mykytyn et al. 2001, 2002; Nishimura et al. 2001, 2005; Otto et al. 2010a; Slavotinek et al. 2000; Stoetzel et al. 2006, 2007; Young et al. 1999). Mutations in known *BBS* genes are found in about 75% of families, of which *BBS1* and *BBS10* each account for 20–25% (Beales et al. 2003; Stoetzel et al. 2006), *BBS12* for about 5% (Stoetzel et al. 2007), and each of the other 12 genes for less than 5% (Chiang et al. 2006; Katsanis 2004) in Caucasians. Second-site phenotypic modification, whereby mutations at a second gene modulate the penetrance and/or expressivity of recessive mutations at a primary locus, has been suggested to play a role in certain cases (Badano et al. 2006; Katsanis et al. 2001; Khanna et al. 2009). Two common mutations have been described: p.M390R in *BBS1* (Mykytyn et al. 2002) and p.C91fsX95 in *BBS10* (Stoetzel et al. 2006). The BBS proteins can roughly be divided into two groups. BBS1, -2, -4, -5, -7, -8 and -9 form a complex called the BBSome, which cooperates with the GTPase Rab8 to promote ciliogenesis (Nachury et al. 2007). A second group is formed by the chaperonin-like proteins BBS6, -10 and -12, which represent a vertebrate-specific branch of the type II chaperonin superfamily (Billingsley et al. 2010; Stoetzel et al. 2007).

The broad genetic locus heterogeneity in BBS renders mutational analysis expensive and time-consuming. Genome-wide homozygosity mapping greatly reduces the number of genes to be sequenced by defining candidate regions of homozygosity by descent, but can only be applied in consanguineous families (Hildebrandt et al. 2009; Lander and Botstein 1987; Nishimura et al. 2005). Here, we report the use of a combined approach of DNA pooling and massively parallel resequencing (MPR) (Otto et al. 2010b) to screen individuals from 105 families from both inbred and outbred backgrounds for mutations in *BBS1–BBS12*. Mutations were assigned to their mutation carrier using CEL I endonuclease heteroduplex screening and confirmed by direct Sanger sequencing (Otto et al. 2008). In 29 out of 105 families (28%), two mutated alleles in 10 different *BBS* genes were identified. In these 29 families, a total of 35 different pathogenic mutations were identified, 18 of which were novel. Two families carried a novel change of uncertain pathogenicity in addition to two mutated alleles. In addition, 12 different single heterozygous mutations, of which 4 have previously been published, were found in 12 out of 105 (11%) families. Thus, DNA pooling combined with MPR offers a valuable strategy for mutation analysis of large patient cohorts of both inbred and outbred backgrounds, especially in heterogeneous diseases such as BBS.

## Patients and methods

### Patients and DNA pooling

DNA samples and clinical information were obtained after receiving informed consent from 132 individuals diagnosed with BBS from 105 different families. The diagnoses were ascertained according to previously established criteria (Baker and Beales 2009). This study was approved by the Internal Review Board of the University of Michigan, the Ethics Committee of the UCL Institute of Child Health and The Internal Review Boards of the Johns Hopkins University and Duke University. Previous mutation screening by linkage and homozygosity mapping in a subset of patients could not identify two mutated alleles in a known *BBS* gene (Harville et al. 2010; Katsanis et al. 2001; Stoetzel et al. 2006). Evidence for consanguinity was found in 16 families after total genome search for linkage was performed in 28/105 families. DNA of 105 families (one affected individual per family) was divided over 5 pools with 21 individuals each. Genomic DNA of 21 individuals was pooled with 2 µg per individual and diluted to 60 ng/µl. In addition, an equimolar DNA pool was generated by pooling 96 DNA samples derived from healthy individuals of Caucasian origin [Human Random Control DNA Panel-1 (HRC-1); European Collection of Cell Cultures, Salisbury, UK].

### PCR amplification and massively parallel resequencing

DNA pools were used as templates to individually amplify all 132 exons of the genes *BBS1–BBS12* by PCR (primer sequences and PCR conditions available upon request). For each pool, PCR products were combined, enzymatically modified and constructed into an Illumina sequencing library as previously described (Otto et al. 2010b). Each library was sequenced using a single lane on an Illumina Genome Analyzer II™ platform generating between about 9 and 26 million reads of 39 bases each. Reads were aligned to the hg18 genomic sequence of the 132 target exons ±100 bp adjacent intronic sequence (<http://genome.ucsc.edu>) using CLC Genomics Workbench software™ (CLC-bio, Aarhus, Denmark).

### Mutation detection and carrier identification

For patient pools #2–4 and the healthy control pool, variant calls were obtained using the following filter parameters: coverage 300×, variant frequency 0.7%, and a minimum variant count of five reads (including duplicate reads). For patient pool #1 and 5, variant calls were obtained using the following filter parameters: coverage 300×, variant frequency

0.9%, and a minimum variant count of five reads (Supplementary Table 1). Variants present in dbSNP130, the “1,000 Genomes Project” (180 control individuals, <http://www.1000genomes.org/page.php>) or in the healthy control pool of 96 individuals (HRC-1) were excluded from further analysis. To prioritize for pathogenic mutations, only variants that were likely to truncate the protein (nonsense, frameshift or obligatory splice site mutations) or missense mutations predicted to be possibly damaging with a PolyPhen score of at least 1.4 were further analyzed. *Polymorphism Phenotyping* (Poly-Phen) is a software tool used to predict the effect of a non-synonymous SNP on protein structure and function (<http://genetics.bwh.harvard.edu/pph/>; Ramensky et al. 2002). These variants were amplified by PCR for each patient in the respective DNA pool and analyzed by either CEL I endonuclease heteroduplex screening (Otto et al. 2008) with subsequent Sanger sequencing or by Sanger sequencing alone to determine the mutation carrier. For all individuals in whom only one mutated allele was discovered, all exons of the respective gene were sequenced by direct Sanger sequencing.

## Results

In an ethnically diverse cohort of individuals diagnosed with BBS, we screened for mutations in the genes *BBS1–BBS12* using a combined approach of DNA pooling and MPR. DNA of 105 individuals was divided over five pools and amplified by PCR. All PCR products of each pool were subjected to MPR on a single lane of an Illumina Genome Analyzer II™ platform. This yielded on average 19,534,601 reads (ranging from 8,995,581 to 25,708,164) of 39 bases in length, of which 77% of all reads mapped back to one of the 132 exons of *BBS1–BBS12* ±100 bp adjacent intronic sequence after alignment to the NCBI36/hg18 human reference sequence. The median coverage for coding nucleotides was 8,554 (mean 19,445) reads, resulting in an average coverage depth of 203 (median 452) reads per site, per single allele. For all five pools, an average of 96% of nucleotides met a 300-fold minimal coverage depth. This translates into an average count of 7 per allele, which is sufficient to call a heterozygous change in 1 out of 42 pooled alleles (21 patients) (Supplementary Table 1).

MPR mutation analysis of all PCR products from 21 individuals in a representative pool (pool #3) yielded a total of 51 non-synonymous variants from normal reference sequence (VRS) (Supplementary Table 1). Of these, 17 were known SNPs and 5 were present in a cohort of 96 Caucasian healthy control individuals and are thought to be as yet unannotated SNPs or sequencing and/or alignment artifacts. Of the remaining 29 changes, 17 changes were predicted to truncate the protein product or affect obligatory splice sites, or had a PolyPhen score higher than 1.4, and were thus assumed to affect the function of the encoded protein. These changes were followed up by CEL I endonuclease heteroduplex screening and subsequent Sanger sequencing, confirming a total of six different changes (Supplementary Table 1). For six cases in which CEL I endonuclease heteroduplex screening did not indicate a mutation carrier, direct Sanger sequencing of all patients in the pool was performed and confirmed one additional change. If initially only one mutated allele was identified in a patient, all exons of the gene involved were sequenced by direct Sanger sequencing in order to find a second mutated allele, which led to the identification of two additional mutations. An analogous approach was applied to the remaining four pools (Supplementary Table 1). In all five pools together, 49 different changes out of 143 possibly damaging changes were confirmed (Supplementary Table 1), of which 28 were novel findings. Of these, 18 mutations are considered to be pathogenic, while 10 changes are of uncertain pathogenicity. This increases the number of mutations known to be involved in BBS from 276 to 294 (6.5%).

Both mutated alleles were identified in 29 out of 105 families (Table 1). The primary genes most frequently involved were *BBS10* (9 families) and *BBS2* (8 families). The other mutated genes were *BBS1* (4 families), *BBS4* (1 family), *BBS5* (1 family), *BBS7* (1 family), *BBS8* (2 families), *BBS9* (2 families) and *BBS12* (1 family). No primary mutations were identified in *BBS3* (*ARL6*), *BBS6* (*MKKS*) or *BBS11* (*TRIM32*). Of the 37 different mutations identified (Table 1), 35 mutations were assumed to be pathogenic, whereas two changes were present as third alleles and of unknown pathogenicity. Of the 35 pathogenic mutations, 18 were novel (Fig. 1). The novel mutations were present in eight different *BBS* genes (*BBS1*, -2, -6, -7, -8, -9, -10, and -12). They are distributed as follows: two nonsense mutations (p.R480X in *BBS2*; and p.Y469X in *BBS10*), six small insertions/deletions leading to a frame-shift (p.L457fsX30 in *BBS2*; p.G162fsX4 and p.I334fsX1 in *BBS8*; p.A672fsX219 in *BBS9*; and p.Q131fsX4 and p.Y559fsX1 in *BBS10*), three obligatory splice site mutations (c.IVS5-1G > C in *BBS1*; c.IVS6+2 in *BBS2*, and c.IVS2+1G > A in *BBS8*) and seven missense mutations (p.G316D in *BBS2*; p.Q293P in *BBS7*; p.L350R in *BBS9*; p.G180E, p.H300P and p.A417V in *BBS10*; and p.G333E in *BBS12*). Of these, 7 were found in the homozygous state and 11 in the compound heterozygous state (Table 1).

A number of mutations were present in more than one family. Families AR14 (Northern Europe) and AR634 (Northern Europe) shared the same novel change in *BBS7* (p.Q293P). In families AR724 (Northern Europe) and AR850 (Northern Europe), the same heterozygous change in *BBS2* (p.Y24X) was present. Likewise, another heterozygous nonsense mutation (p.R275X) in *BBS2* was shared between families A1885 (USA) and A2296 (USA). Affected individuals from both families AR122 (Northern Europe) and AR603 (Northern Europe) carried the same compound heterozygous mutations in *BBS1* (p.R146X and p.M390R) (Beales et al. 2003). The previously described recurrent mutation in *BBS1*, p.M390R (Mykytyn et al. 2002) was present in the heterozygous state in two additional families, AR61 and AR786. A recurrent mutation in *BBS10*, p.C91fsX95 (Stoetzel et al. 2006) was present in eight families. This change was present in the homozygous state in one family and as a heterozygous change in seven families, in which case the second mutated allele in *BBS10* was consistently found. Two affected individuals from family AR61 carried three heterozygous changes in *BBS1*, two of which have previously been published (p.L75fsX23, p.M390R) (Beales et al. 2003; Mykytyn et al. 2002), while one is novel (p.L206V) and of uncertain pathogenicity. In family AR850, a missense change in *BBS6* (p.I432F) with a moderate PolyPhen score (1.523) was present in addition to compound heterozygous mutations in *BBS2* (p.Y24X, p.L168fsX33) in one affected individual (AR850-04), but not in the second affected sibling (AR850-03) (Table 1).

In addition to these families in which at least two mutated alleles in one *BBS* gene were found, 12 different single heterozygous changes of uncertain pathogenicity have been identified in 12 different families (Table 2). These cover the genes *BBS1*, -2, -4, -5, -6, -7, -9, -10 and -12, and are all missense mutations, four of which have previously been published. In family A2499, two novel heterozygous missense mutations were present in two different genes: *BBS1* (p.P245L) and *BBS9* (p.L781Q). The affected individual from family A3227 carried one novel heterozygous missense change in *BBS10* (p.R530S) and one in *BBS12* (p.V503M).

In all cases where DNA from relatives was available, mutations segregated as expected. All changes stated were absent from 96 Caucasian healthy control individuals and were not present in 180 control individuals from the “1,000 Genomes Project” (<http://www.1000genomes.org/page.php>).



## Discussion

Conventional mutation analysis is tedious, and expensive to perform in diseases that exhibit broad genetic locus heterogeneity such as BBS. Most previous large scale mutation analysis studies focused solely on a subset of the known BBS loci (Billingsley et al. 2010; Hjortshøj et al. 2010; Muller et al. 2010). Here, we present the application of a DNA pooling and massively parallel resequencing strategy that we developed recently (Otto et al. 2010b) to identify mutations in patients with BBS. We estimate the cost of this MPR mutation analysis to be in the range of \$50–60 per patient. We discovered the disease-causing mutations in 29 out of 105 families (28%). In total, we identified 49 different mutations, 28 of which are novel. Of these, we consider 18 mutations to be pathogenic, while 10 changes are of uncertain pathogenicity. We thereby increase the number of known causative BBS mutations from 276 to 294 (6.5%).

In this study, we were able to identify both disease causing alleles in 28% of our patient cohort. In contrast, Muller et al. 2010 identified both disease causing alleles in 67% of their patient cohort. This discrepancy is most likely due to the fact that the majority of our cohort has previously been screened for homozygous mutations by linkage and homozygosity mapping (Harville et al. 2010; Katsanis et al. 2001; Stoetzel et al. 2006). Furthermore, this may reflect potential differences in the sensitivity or specificity of sequencing technology: our method might miss mutations, if they are positioned in an area of low coverage (<300 reads) or if they are insertions or deletions larger than 2 bp in length. We did not search for copy-number variants (CNV's) to detect large heterozygous deletions. Mutations such as complete exon deletions, promoter mutations, or intronic exon splice enhancers (ESE) are not detected by our method.

In one family (AR850), we found a third mutated allele in addition to the primary BBS locus which could represent a form of oligogenic inheritance (Badano et al. 2003b). However, this potential modifier allele is a missense mutation of unknown pathogenicity and functional studies are required in order to draw conclusions about its potential modifying effects. In family AR850, the p.I432F allele in *BBS6* was only present in one of the two affected siblings who exhibit differences in secondary symptoms (Table 1). It has recently been shown that variants predicted in silico to be neutral, can act as modifiers to exacerbate phenotypes across the ciliopathy spectrum (Badano et al. 2003b; Khanna et al. 2009; Zaghoul et al. 2010). However, we did not investigate the presence of these variants in our patient cohort. Due to the relatively high false positive error rate in next generation sequencing, we prioritized our analysis to detect mutations that are predicted to functionally damage the encoded proteins.

Our samples (pool #1–5) were sequenced successively over a period of 6 months, during which a clear improvement of next-generation sequencing was observed, with an error rate reduction due to higher coverage. The number of false positive variants in the pool sequenced first (pool #1) [29/43 (67%)] was remarkably higher than in the pool sequenced 6 months later (pool #5) [1/12 (8%)] (Supplementary Table 1). This considerable decrease in false positive variants in pool #5 allowed us to assign variants to their mutation carrier by direct Sanger sequencing alone, without tedious CEL I endonuclease heteroduplex screening. In addition, the number of variants that we discovered upon sequencing of the whole gene if a first mutation was found in a family (“false negative variants”) decreased over the course of our study, with 3 false-negatives in pool # 1 [3/43 (7%)] and none in pool #5 [0/12 (0%)]. Future application of paired-end technology would reduce the number of false-positive and negative calls even further, which would be of major importance for clinical utility. Other technological improvements to next-generation sequencing such as increasing read length and read number will also substantially cut down on the number of

false-positive and negative variants detected. This will enable comprehensive, robust, rapid and cost effective mutation analysis for many patients across genetically heterogeneous diseases.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

<b>BBS</b>	Bardet–Biedl Syndrome
<b>bp</b>	Base pairs
<b>GTP</b>	Guanosine triphosphate
<b>HRC-1</b>	Human random control DNA panel-1
<b>MPR</b>	Massively parallel resequencing
<b>nt</b>	Nucleotides
<b>PCR</b>	Polymerase chain reaction
<b>VRS</b>	Variants from reference sequence

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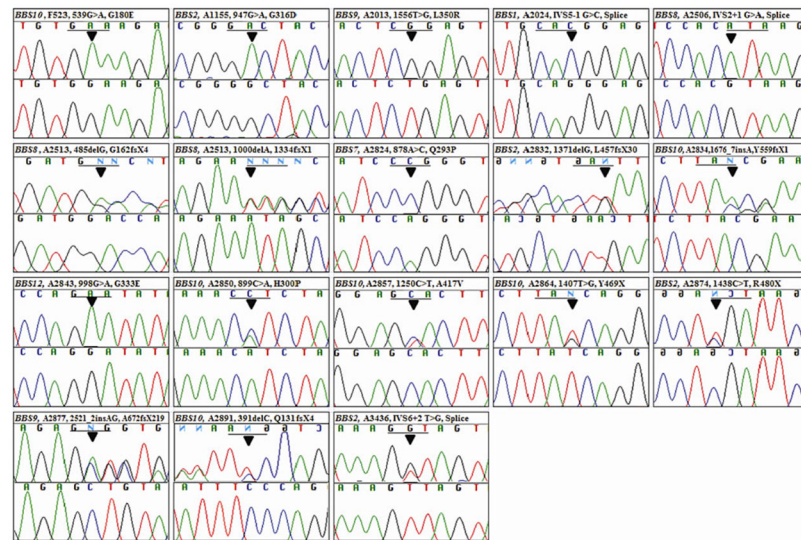
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**Fig. 1.** Sequence chromatograms of 18 different novel mutations identified in individuals with BBS. Gene name, patient identifier, nucleotide change, and inferred amino acid alteration are given above sequence traces. The mutation position is indicated with an *arrow*. Wild type sequence chromatograms are shown below mutated sequences. Reading frames are *underlined*

Genotypes and phenotypes of 39 individuals with BBS (29 families) with both mutated alleles detected in one of 12 known *BBS1–BBS12*

Table 1

Family (Individual (alias))	Cardinal features				Secondary signs	Origin	<i>BBS</i> gene <sup>d</sup>	Nucleotide change (zygosity state) <sup>b</sup>	Amino acid change (segregation)	Count/coverage (% frequency)	Mutation assignment method <sup>c</sup>	Mutation reference	PolyPhen PSIC score
	Retinitis pigmentosa	Postaxial polydactyly	Obesity	Hypogonadism									
PB206 (A2024)	-III ND	Y	Y	Y	DD, MR	Turkey	1	c.1VSS-1 G>C (H)	Splice site	891/24,085 (3.7%)	All pat seq	Present study	n/a
AR61 (A2826)	-04 Y	N	Y	N	LD, UTIS	N. Eur.	1	c.223_224delCT (h)	p.L75fsX23	85/9,054 (0.9%)	CEL 1	Beales et al. 2003	n/a
	-05 Y	Y	Y	N	LD, SD		1	c.1169T>G (h)	p.M390R	–	All exon seq	Mykytyn et al. 2002	2.703
AR122 (A2831) <sup>d</sup>	-04 Y	N	Y	Y	MR	N. Eur.	1	c.616T>G (h)	p.L206V	–	All exon seq	Present study	1.099
							1	c.436C>T (h)	p.R146X	24/1,589 (1.5%)	CEL 1	Beales et al. 2003	n/a
AR603 (A2858)	-03 Y	Y	Y	N	LD, PP, SD	N. Eur.	1	c.1169T>G (h)	p.M390R	–	All exon seq	Mykytyn et al. 2002	2.703
	-05 Y	Y	Y	ND	ND		1	c.436C>T (h)	p.R146X	10/1,119 (0.9%)	All pat seq	Beales et al. 2003	n/a
A1155	-III N	Y	ND	N	ASD	N. Eur.	2	c.1169 T>G (h)	p.M390R	–	All exon seq	Mykytyn et al. 2002	2.703
A1885	-III Y	N	ND	ND	–	USA	2	c.947G>A (H)	p.G316D	235/6,551 (3.6%)	All pat seq	Present study	2.158
							2	c.823C>T (h)	p.R275X	787/5,356 (14.7%)	All pat seq	Katsanis et al. 2001	n/a
A2296	-III N	Y	ND	ND	ASD, IVS-AZ, SI	USA	2	c.1895G>C (h)	p.R632P	232/23,796 (1.0%)	All pat seq	Katsanis et al. 2001	2.698
AR124 (A2832)	-03 Y	Y	Y	N	DD	N. Eur.	2	c.823C>T (H)	p.R275X	787/5,356 (14.7%)	All pat seq	Katsanis et al. 2001	n/a
	-04 Y	Y	Y	Y	DD		2	c.1VSS-1delG (h)	p.V158fsX43 <sup>e</sup>	6/526 (1.1%)	All pat seq	Katsanis et al. 2001	n/a
AR724 (A2866)	-05 Y	Y	ND	Y	DD	N. Eur.	2	c.1371delG (h)	p.L457fsX30	–	All exon seq	Present study	n/a
					BCU, HMC, MD		2	c.175C>T (h)	p.Q59X <sup>f</sup>	72/7,990 (0.9%)	CEL 1	Katsanis et al. 2001	n/a
AR839 (A2874)	-04 Y	Y	Y	N	CHD, MD, NSM, SD, SI	N. Eur.	2	c.72C>G (h)	p.Y24X	–	All exon seq	Katsanis et al. 2001	n/a
							2	c.1438C>T (h)	p.R480X	13/338 (3.6%)	CEL 1	Present study	n/a
AR850 (A2878)	-03 Y	Y	Y	Y	AST, DD, MC, SD, SYN	N. Eur.	2	c.1237C>T (h)	p.R413X	97/5,450 (1.8%)	CEL 1	Fausser 2003	n/a
	-04 Y	Y	Y	Y	MC, SD		2	c.72C>G (h)	p.Y24X	198/27,120 (0.7%)	CEL 1	Katsanis et al. 2001	n/a
							2	c.504delG (h)	p.L168fsX33	12/1,603 (0.7%)	CEL 1	Muller et al. 2010	n/a
A3436	-III N	Y	Y	ND	–	Turkey	6	c.1294A>T (h) <i>only in individual -104</i>	p.I432F	137/8,059 (1.7%)	CEL 1	Present study	1.523
KK44 (A2884)	-08 Y	Y	Y	Y	–		2	c.940delA (h)	p.I314fsX11 (p)	59/6,577 (0.9%)	All pat seq	Nishimura et al. 2001	n/a
A2507	Y	Y	Y	Y	–	Saudi Arabia	4	c.1VSG+2 (h)	Splice site (m)	69/8,574 (0.8%)	All pat seq	Present study	n/a
AR14 (A2824)	-04 Y	Y	Y	Y	AN, FL, MR, PS	Somalia	5	c.1VSS-2A>G (H)	Splice site	84/4,953 (1.7%)	CEL 1	Katsanis et al. 2002	n/a
	-08 Y	Y	Y	Y	MR	N. Eur.	7	c.214 G>A (H)	p.G72S	45/2,423 (1.9%)	CEL 1	Hjortshøj et al. 2008	2.112
					MR, PN			c.878A>C (H)	p.Q293fs <sup>g</sup>	261/5,626 (4.6%)	All pat seq	Present study	2.167
A2506	Y	Y	Y?	N	AZ, MR, NE	ND	8	c.1VSS-1 G>A (H)	Splice site	393/9,334 (4.2%)	CEL 1	Present study	n/a
A2513	Y	Y	Y	Y	FL, GASTS, MR	Hispanic	8	c.485delG (h)	p.G162fsX4	47/1,946 (2.4%)	CEL 1	Present study	n/a

Family (Individual [alias])	Cardinal features			Secondary signs	Origin	BBS gene <sup>d</sup>	Nucleotide change (zygosity state) <sup>b</sup>	Amino acid change (segregation)	Conti/coverage (% frequency)	Mutation assignment method <sup>c</sup>	Mutation reference	PolyPhen PSIC score
	Retinitis pigmentosa	Postaxial polydactyly	Obesity	Hypogonadism	Renal anomalies							
PB233 (A2013)	-III	Y	Y	Y	ND	8	c.1000delA (h)	p.L334fsX1	–	All exon seq	Present study	n/a
	-II2	ND	Y	ND	ND	9	c.1556T>G (H)	p.L350R	163/4,646 (3.5%)	All pat seq	Present study	1.885
AR847 (A2877)	-03	Y	Y	N	Y	9	c.2390_2393delAACAA (h)	p.K628fsX222 <sup>b</sup>	–	All exon seq	Nishimura et al. 2005	n/a
F523	-04	Y	Y	N	Y	9	c.2521_2522insAG (h)	p.A672fsX219	14/930 (1.5%)	All pat seq	Present study	n/a
	-III	ND	ND	ND	Y	10	c.539G>A (H)	p.G180E	603/15,920 (3.8%)	All pat seq	Present study	2.172
AR151 (A2834)	-04	Y	Y	N	ND	10	c.271_272msT (h)	p.C91fsX5	33/1,310 (2.5%)	CEL 1	Stoetzel et al. 2006	n/a
AR232 (A2839)	-03	Y	Y	Y	N	10	c.1676_1677msA (h)	p.Y559fsX1	5/604 (0.8%)	CEL 1	Present study	n/a
	-04	Y	Y	Y	N	10	c.1677delC (h)	p.Y559fsX1 (m) <sup>d</sup>	8/673 (1.2%)	CEL 1	Stoetzel et al. 2006	n/a
AR371 (A2850)	-03	Y	Y	Y	ND	10	c.271_272msT (h)	p.C91fsX5 (p)	33/1,310 (2.5%)	CEL 1	Stoetzel et al. 2006	n/a
	-03	Y	Y	N	ND	10	c.271_272msT (h)	p.C91fsX3 <sup>d</sup>	64/1,396 (4.6%)	CEL 1	Stoetzel et al. 2006	n/a
AR515 (A2857)	-03	Y	Y	Y	N	10	c.899A>C (h)	p.H300P	2,056/119,460 (1.7%)	CEL 1	Present study	2.575
AR707 (A2864)	-08	Y	Y	N	Y	10	c.271_272msT (h)	p.C91fsX5	64/1,396 (4.6%)	CEL 1	Stoetzel et al. 2006	n/a
	-09	Y	Y	N	Y	10	c.1250C>T (h)	p.A417V	–	All exon seq	Present study	1.413
AR880 (A2881)	-04	Y	Y	N	Y	10	c.271_272msT (h)	p.C91fsX5 <sup>d</sup>	198/5,633 (3.5%)	CEL 1	Stoetzel et al. 2006	n/a
F1 (A2891)	-04	Y	Y	Y	Y	10	c.271_272msT (h)	p.C91fsX5	451/23,019 (2.0%)	CEL 1	Present study	n/a
A3185	-05	Y	Y	Y	Y	10	c.391delC (h)	p.Q131fsX4	64/1,396 (4.6%)	CEL 1	Stoetzel et al. 2006	n/a
	-III	Y	Y	Y	Y	10	c.687delA (h)	p.P229fsX8	198/5,633 (3.5%)	All pat seq	Stoetzel et al. 2006	n/a
AR248 (A2843)	-05	Y	Y	Y	Y	12	c.271_272msT (h)	p.C91fsX5	1,036/78,357 (1.3%)	All pat seq	Present study	n/a
						10	c.687delA (h)	p.P229fsX8	750/55,490 (1.4%)	All pat seq	Stoetzel et al. 2006	n/a
						10	c.271_272msT (h)	p.C91fsX5	203/16,449 (1.2%)	All pat seq	Stoetzel et al. 2006	n/a
						12	c.998G>A (H)	p.G333E	193/14,768 (1.3%)	All pat seq	Present study	2.274

*AMRR* amenorrhea, *AN* anosmia, *ASD* atrial septal defect, *AST* astigmatism, *AZ* asthma, *BCU* bicornuate uterus, *BD* brachydactyly, *BE* bifid epiglottis, *CHD* congenital heart disease, *COM* chronic otitis media, *Cons* constipation, *CTEV* congenital talipes equinovarus, *DD* developmental delay, *FL* fatty liver, *GASTS* gall stones, *h* heterozygous change, *HAP* high arched palate, *HMC* hydrometrocolpos, *HT* hypotonia, *IM* intestinal malrotation, *IVC-AZ* inferior vena cava interruption with azygos continuation, *LD* learning disabilities, *MC* macrocephaly, *MD* motor delay, *MR* mental retardation, *MY* (severe) myopia, *n/a* not available, *ND* no data, *NE* nasal encephalocoele, *N. Eur.* Northern Europe, *NSM* narrow small mouth, *PN* peripheral neuropathy, *PP* precocious puberty, *PS* psychosis, *Ri* rickets, *Scol* scoliosis, *SD* speech delay, *SDys* sensory dysesthesia, *SJ* situs inversus, *SM* splenomegaly, *TCP* thrombocytopenia, *UTIS* recurrent urinary tract infections

<sup>a</sup>Accession numbers: *BBS1*, NM\_024649; *BBS2*, NM\_031885; *BBS3*, NM\_177976; *BBS4*, NM\_033028; *BBS5*, NM\_152384; *BBS6*, NM\_018848; *BBS7*, NM\_176824; *BBS8*, NM\_198309.2; *BBS9*, NM\_198428; *BBS10*, NM\_024685; *BBS11*, NM\_012210; *BBS12*, NM\_152618

<sup>b</sup>Mutation numbering is based on the cDNA position in reference sequences indicated in Table 1 with +1 corresponding to the A of the ATG translation initiation codon. All changes were absent from the healthy control pool (96 HRC-1 individuals) and the “1,000 genomes project” (180 individuals)

<sup>c</sup>The mutation carrier assignment was performed by heteroduplex based CEL 1 endonuclease screening (CEL 1) or by direct Sanger sequencing of the respective 21 DNA samples (All pat seq). If initially only one mutated allele was found, all exons of the respective gene were sequenced (All exon seq)

<sup>d</sup>Family AR122 (A2831) has previously been published for linkage to the *BBS1* locus by Katsanis et al. 1999



<sup>e</sup>Family AR124 (A2832) has previously been published for this heterozygous change in *BBS2* (p.V158fsX43) by Katsanis et al. 2001

<sup>f</sup>Family AR724 (A2866) has previously been published for this heterozygous change in *BBS2* (p.Q59X) by Katsanis et al. 2001

<sup>g</sup>Family AR14 (A2824) has previously been published for a heterozygous change in *BBS12* (p.G540D) present in individual '-04' (but not '-08') by Stoetzel et al. 2007

<sup>h</sup>The mutation p.K628fsX22 has previously been published by Nishimura et al. 2005 as p.K626fsX647

<sup>i</sup>Family AR232 (A2839) has previously been published for this heterozygous change in BBS10 (p.Y559fsX1) by Stoetzel et al. 2006

<sup>j</sup>Family AR371 (A2850) and AR707 (A2864) have previously been published for this heterozygous change in BBS10 (p.C91fsX5) by Stoetzel et al. 2006

Genotypes and phenotypes of 14 individuals with BBS (12 families) with only one mutated allele detected in a known *BBS1*–*BBS12*

Table 2

Family [Individual (alias)]	Cardinal features				Secondary signs	Origin	<i>BBS</i> gene <sup>d</sup>	Nucleotide change (zygosity state) <sup>b</sup>	Amino acid change (segregation)	Count/coverage (% frequency)	Mutation assignment <sup>c</sup>	Mutation reference	PolyPhen PSIC score
	Retinitis pigmentosa	Postaxial polydactyly	Obesity	Hypogonadism									
A2499	Y	Y	N	N	–	ND	1	c.734 C>T (h)	p.P245L	6/382 (1.6%)	All pat seq	Present study	2.328
A2517	Y	Y	N	N	DD, HAP, OB	ND	9	c.2849 T>A (h)	p.L781Q	4/425 (0.9%)	CEL_1	Present study	1.885
AR786 (A2868)	–03	N	Y	ND	MC, MD, MR, SD	N. Eur.	1	c.871 C>T (h)	p.Q291X	1,607/54,862 (2.9%)	CEL_1	Beales et al. 2003	n/a
–04	Y	Y	ND	ND	MR, SD, SDys		1	c.1169 T>G (h)	p.M390R	2/22 (9.1%)	All pat seq	Mykyryn et al. 2002	2.703
PB236 (A2010)	–III	Y	Y	ND	ATX, BD, STBI	Turkey	2	c.1891 G>A (h)	p.A631T	10/494 (2.0%)	All pat seq	Present study	1.522
AR348 (A2848)	–03	Y	Y	ND	–	N. Eur.	4	c.218A>G (h)	p.Q73R	74/3,159 (2.3%)	CEL_1	Present study	1.574
AR364 (A2849)	–02	Y	Y	Y	DD, HNP	N. Eur.	5	c.551A>G (h)	p.N184S	41/1,353 (3.0%)	All pat seq	Li et al. 2004	2.211
AR755 (A2867) <sup>d</sup>	–03	Y	Y	ND	BD, DD, DF, SD	N. Eur.	5	c.551A>G (h)	p.N184S	41/1,353 (3.0%)	All pat seq	Li et al. 2004	2.211
A786	–III	N	Y	Y	ASD, MR, MY, Sz	Macedonia	6	c.724G>T (h)	p.A242S	216/12,651 (1.7%)	All pat seq	Stone et al. 2000	1.280
AR800 (A2870)	–03	Y	Y	ND	MR, SS, STBI	N. Eur.	6	c.724G>T (h)	p.A242S	1,878/55,174 (3.4%)	CEL_1	Stone et al. 2000	1.280
AR634 (A2862)	–III	Y	Y	Y?	DD, MR, SD	N. Eur.	7	c.878A>C (h)	p.Q293P	26/15,626 (4.6%)	All pat seq	Present study	2.167
–II2	Y	N	Y	Y	MC, MD, SS								
A3260	–III	N	Y	N	PSp, SI	USA	9	c.2983C>T (h)	p.R826C	1,575/25,567 (6.2%)	All pat seq	Present study	2.257
A3227	–III	N	Y	N	BD, MR, PHP, SS	Egypt	10	c.1590A>C (h)	p.R530S	102/11,606 (0.9%)	All pat seq	Present study	1.686
							12	c.1507G>A (h)	p.V503M	509/37,380 (1.4%)	All pat seq	Present study	1.430

*ASD* atrial septum defect, *ATX* ataxia, *BD* brachydactyly, *DD* developmental delay, *DF* dysmorphic features, *HAP* high arched palate, *HNP* herniated nucleus pulposus, *MC* macrocephaly, *MD* motor delay, *MR* mental retardation, *MY* (severe) myopia, *OB* overbite, *PHP* pseudohypoparathyroidism, *PSp* polysplenia, *SD* speech delay, *SDys* sensory dysesthesia, *SI* situs inversus, *SS* short stature, *STBI* strabismus, *Sz* seizures, *ND* no data available, *N. Eur.* Northern Europe

<sup>a</sup>Accession numbers: *BBS1*, NM\_024649; *BBS2*, NM\_031885; *BBS3*, NM\_177976; *BBS4*, NM\_033028; *BBS5*, NM\_152384; *BBS6*, NM\_018848; *BBS7*, NM\_176824; *BBS8*, NM\_198309.2; *BBS9*, NM\_198428; *BBS10*, NM\_024685; *BBS11*, NM\_012210; *BBS12*, NM\_152618

<sup>b</sup>Mutation numbering is based on the cDNA position in reference sequences indicated in Table 1 with +1 corresponding to the A of the ATG translation initiation codon. All changes were absent in the healthy control pool (96 HRC-1 individuals) and the “1,000 genomes project” (180 individuals)

<sup>c</sup>The mutation carrier assignment was performed by heteroduplex based CEL 1 endonuclease screening (“CEL I”) or by direct Sanger sequencing of the respective 21 DNA samples (“All pat seq”). If initially only one mutated allele was found, all exons of the respective gene were sequenced (“All exon seq”)

<sup>d</sup>Unpublished mutation analysis of family AR755 (A2867) showed the presence of a homozygous change in *BBS1* (p.M390R)